APOGAMY IN NEPHRODIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY XCVIII

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This preliminary note will give a brief account of my cytological studies on apogamy in *Nephrodium molle* Desv., which were conducted at the Hull Botanical Laboratory, the University of Chicago, under the direction of Professor JOHN M. COULTER and Dr. CHARLES J. CHAMBERLAIN, to whom I acknowledge my great indebtedness for their suggestion and assistance in this investigation.

To undertake the solution of such an important problem as apogamy, it is absolutely necessary for an investigator to have beforehand a thorough knowledge of the nuclear condition in the normal life-history of a selected form. For this reason I have made a complete study of the normal life-history of Nephrodium in order to obtain the knowledge requisite for an investigation of the problem of apogamy.

The detailed account of this investigation, with plates and a discussion of literature, will be published later in three successive papers, entitled "Sporogenesis;" "Spermatogenesis, oogenesis, and fertilization;" and "A study of apogamy."

Mitoses in the sporophyte.—It was very easy to get the successive stages of the process of the vegetative mitoses in various parts of the leaf, but the most favorable figures were obtained during the formation of the sporogenous tissue.

The resting nucleus in the sporogenous tissue contains a delicate network with one or several nucleoli. Approaching the prophase of the mitosis the network passes into the continuous spirem, uniform in thickness and distributed throughout the nuclear cavity. Two kinoplasmic caps with few fibers are formed at the two poles outside the nucleus. The nuclear membrane becomes elongated a little toward the kinoplasmic caps and begins to break down at that point. Coincident with this the spirem segments into a number of chromosomes to which intruding spindle fibers become attached and finally establish an equatorial plate that has a characteristic aspect which will be shown in a final paper.

The chromosomes at the equatorial plate split longitudinally, and, after a peculiar change of their form, two groups of daughter chromosomes pass to the opposite poles of the spindle, where they become closely crowded in a mass. The polar view of this stage shows that the number of chromosomes is 128 or 132. Nucleoli fuse usually into one or two globules which decrease in size, finally vanishing during the prophase. The daughter chromosomes, crowded in a mass at the poles of the spindle, become so closely aggregated that it is hard to identify their individual outlines; then follows vacuolization of each chromosome.

The central spindle lying between two poles after organization of the daughter nuclei seems to contribute material for the formation of the cell plate, and consequently it proceeds from the center toward the periphery of the cell.

The resting nucleus of the spore mother cell contains a fine network of linin, in which the chromatin material is distributed in larger and smaller knots. With the further growth of the nucleus, the knots in the network decrease in size, whereas the delicate linin part increases in thickness, and finally there is formed a well-developed spirem, uniform in thickness and very much tangled. This state soon passes into the synaptic stage, which seems to culminate by location of the tangled spirem at one side of the nuclear cavity. The spirem consists of two threads, close together; in some parts the two are in contact side by side, and in the other parts they are fused into a single thread. These two threads may represent, according to the recent interpretation of synapsis, chromatin of maternal and paternal origin.

After synapsis, the tangled thread begins to uncoil and becomes distributed throughout the nuclear cavity; the spirem shows longitudinal splitting, but the two elements of the spirem which resulted therefrom remain closely associated so as to form a double thread, which finally segments into 66 or 64 chromosomes, each showing clearly its bivalent nature.

Fibrillar structures which first appear at the periphery of the clear region closely surrounding the nucleus become more and more pronounced until they approach the nuclear membrane, and when the membrane breaks down the fibers enter into the nuclear cavity from several directions.

When the spindle becomes bipolar, the chromosomes at the equatorial plate are arranged regularly, so that the counting of their number is easily done from the polar view. These 64 or 66 bivalent chromosomes separate and two sets of daughter chromosomes, each 64 or 66, pass to the poles of the spindle, and daughter nuclei are formed by the vacuolization of chromosomes.

Granularization of cytoplasm now begins at the periphery of the equatorial plate and proceeds toward its center, and seems to replace entirely the central spindles, so that the spore mother cell is divided into two hemispheres by the equatorial zone of granular cytoplasm.

Except in the number of chromosomes, the second, the so-called homotypic mitosis, is essentially the same in Nephrodium as the typical mitosis of the vegetative cell; 64 or 66 chromosomes reappear at the prophase of the second mitosis, split longitudinally, and are distributed into spores.

Spermatogenesis.—The mitoses in the vegetative cells of prothallia are similar to those of the sporophyte. The number of chromosomes, however, in this case is 66 or 64. The account of the mitoses which take place from the cutting-off of an antheridial initial from the superficial cell of the prothallia to the formation of sperm grandmother cells will be omitted. The number of chromosomes is always 66 or 64.

The sperm grandmother cell is characterized by the first appearance of blepharoplasts. The blepharoplasts first appear as two small granules at opposite sides of the nucleus, near the periphery of the cell, which means that the origin of the blepharoplast in Nephrodium is cytoplasmic. The resting nucleus of the sperm grandmother cell passes through the prophase, metaphase, anaphase, and telophase, the number of chromosomes being 66 or 64, and the blepharoplasts are always present near the poles of the spindle. After telophase, each daughter cell of the sperm grandmother cell or the sperm mother cell contains a single blepharoplast.

When the cell plate is completed, a new body appears near the nucleus of the sperm mother cell, far apart from the blepharoplast. For the sake of convenience, we shall term this body, at present, the *Nebenkern.*

The blepharoplast in close contact with the nucleus increases

in size and assumes gradually a coiled band shape, while the nucleus, after an interesting modification of chromatin material, becomes also a coiled structure. The cilia appear only on the outer surface of the blepharoplast. The *Nebenkern* remains attached at the posterior end of the mature sperm, with the remnant of cytoplasmic material.

Oogenesis and fertilization.—In the formation of an archegonial initial from a vegetative cell of the prothallium, 64 or 66 chromosomes are present. The mitoses which occur in the formation of the central cell, neck and ventral cells, and neck canal, ventral canal, and egg cells, have very interesting peculiarities which will be described in a later paper. The noteworthy thing is that the number of chromosomes is 64 or 66.

Sperms pass through the archegonial neck and reach the egg cell. Normally a single sperm succeeds in entering, through the cytoplasm, into the egg nucleus which is in the resting condition. Within the egg the sperm finally loses its individual outline.

The fertilized egg nucleus in the resting state passes into the prophase of the first segmentation division. The spirem is a homogeneous thread structure, uniform in thickness, and seems to be continuous. It segments into a number of chromosomes that become arranged in an equatorial plate. The chromosomes in the equatorial plate split longitudinally and two sets of daughter chromosomes separate and pass toward the poles of the spindle. The polar view of this set shows that the number of chromosomes is 128 or 132.

The further processes of segmentation divisions during the development of an embryo were traced out in order to make certain the number of chrosomomes, and 128 or 132 are always present.

Apogamy.—The prothallia which produce apogamous sporophytes do not seem to be different from those which form normal embryos, so long as they remain a single cell layer in thickness throughout. The morphological structure does not seem to show any peculiarity. Numerous antheridia are produced. Although the mitoses which give rise to sperm mother cells from a primary spermatogenous cell are different from those of a normal case, yet the motile sperms are developed.

The most important and peculiar feature of the prothallia in connection with apogamous growth is as follows: The successive

cell divisions proceed in the cushion region as usual in a normal case, but no functional archegonium is formed. In a certain portion in or near the cushion region, a superficial cell divides perpendicular to the surface of prothallium, while cells below the surface divide in various directions. Thus a mass of peculiar structure, whose further development would result later in an independent sporophyte, is worked out directly by the continuous growth of the prothallium.

The nuclear conditions were traced through the critical stage, intermediate between gametophytic prothallia and sporophytic structure, to the independent sporophyte. The number of chromosomes remains unchanged, always being 64 or 66. There is no migration of a nucleus from a neighboring cell to effect fusion, as is reported in Lastrea.

Conclusions.—The nuclear condition of *Nephrodium molle* may be summarized as follows:

1. The nuclei of the prothallia contain 64 or 66 chromosomes, the reduced, gametophytic, or x number. The nuclei of the gametes contain the same number. The fusion nucleus in the fertilized egg presents 128 or 132 chromosomes, the unreduced, sporophytic, or $2x$ number, which keeps unchanged until it is reduced during sporogenesis. Consequently it follows in the normal life-history of Nephrodium that the gametophyte contains the x number of chromosomes and the sporophyte the $2x$ number, and that sporogenesis and fertilization are the periods which mark the initiation of the two distinct generations.

2. The nucleus of a prothallial cell with the x number of chromosomes (64 or 66) sometimes becomes directly the nucleus of a sporophyte, apogamously produced; so that the x number of chromosomes continues through the whole life-history in the apogamous sporophyte. This fact does not seem to affect the fundamental idea that the alternation of generations is marked by the difference in the number of chromosomes in the normal life-history; but is simply an abnormal case of secondary importance. Still it must be admitted that in the case of apogamy at least the number of chromosomes is not the only factor which determines the character of the sporophyte and gametophyte.

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